United States Department of Agriculture

Food Safety and Inspection Service

To: See DISTRIBUTION

12 JUN 1986

From: Robert C. Post, Food Technologist
Nutrition Branch
Food Ingredient Assessment Division
Science Program

Subject: Concurrence on the Final Version of the "Scheme and Critical Variables for a Limited Study on the Effects of Vacuum-Packaging and Irradiation on the Outgrowth and Toxin Production of Clostridium botulinum in Pork Loins"

As previously indicated, the above captioned document was completed without further revisions required after the subgroup's last meeting on May 19, 1986. I am requesting concurrence from subgroup members so that we can release the document to petitioners and other interested parties. The document and concurrence sheet are enclosed.

DISTRIBUTION:
Robert Post, FIAD, Science
Ralph Johnston, Micro., Science
Dan Engeljohn, PPID, MPITS
Don Kauter, Micro., FDA
Doug Archer, Micro., FDA

cc:
Don Derr, FIAD, Science

Scheme and Critical Variables for a Limited Study on the Effects of Vacuum Packaging and Irradiation on the Outgrowth and Toxin Production of *Clostridium botulinum* in Pork Loins

BACKGROUND:

When a vacuum-packaged fresh pork product has been irradiated between 30-100 Krad, there is concern that a potential health hazard might result from further reducing the natural flora and allowing *Clostridium botulinum* spores, if present, to produce toxin under conditions where typical spoilage is not evident (i.e., before organoleptic indication of spoilage).

After careful review of the scientific literature by FSIS scientists, outside consultants, and the FDA, it was determined that data are needed to support the microbiological safety of irradiated vacuum-packaged pork at an absorbed dose of 30 to 100 Krad. In order to permit this procedure for fresh pork in commerce, under the statutes by which we operate, the Agency must be provided with the necessary data to prove the microbiological safety of irradiation under 100 Krad for vacuum-packaged pork. The scheme (Figure 1) for a limited study to provide the needed data has been developed. The suggested experimental design represents the minimum requirements necessary to fulfill our data needs. Included are critical variables which must be addressed. These critical variables are as follows:

Processor

Pork Supply and Handling Prior to Irradiation:

- Fresh boneless pork loins are to be derived from animals within 48 hours of slaughter from a commercial establishment. They are to be derived from carcasses held at 0°C or less and be reasonably uniform in weight (approximately +/-10 percent).
- Derived loins will be promptly tested to verify they are free from antibiotics, and then sliced.
- Temperature of loins at all times will be approximately 0°C.
- Loins must be sent to the laboratory and inoculated within 48 hours after removal from carcasses.

Competent Laboratory*

Inoculum & Inoculation Procedures:

- Spores are to be produced from five type A and five type B strains of *Clostridium botulinum*.

*must demonstrate in the study protocol that the laboratory is capable of handling botulnal toxin.

- After enumeration of each individual spore crop, crops are to be composited to yield equal spore levels of each strain.

- Just prior to inoculation, the composite spore crop shall be heat shocked by heating the spore suspension to 80°C for 10 minutes.

- Inoculation of product shall be at a level of 50 spores per gram.

- Inoculation of product shall be performed by applying 0.1 ml of composite spore suspension onto the palm of a glove and rubbing this over the product to uniformly distribute the spores.

- Uninoculated and inoculated sliced loins will be vacuum-packaged (without nitrogen flush)--1 sliced loin per package.

- Appropriate numbers of vacuum-packaged uninoculated and inoculated units will be prepared and boxed, and all boxes will be identified, including intended treatment identification. The number of loins per box and an approximation of the number of chops per loin or thickness of the chops should also be identified. Product will be labeled to indicate that it is for experimental purposes.

- Vacuum-packaged uninoculated and inoculated sliced loins will be held and shipped at approximately 0-2°C (not frozen).

- Irradiation of vacuum-packaged uninoculated and inoculated sliced loins will be within 48 hours of inoculation.

Irradiation Facility

- Uninoculated and inoculated sliced loins will be irradiated (within 48 hours after inoculating) at 30, 100, 300, and 500 Krad minimum absorbed dose. The minimum absorbed dose should be within +/-5 percent of the target dose. Processors must define the range actually received by the product and record the dosimetry variation within the 4 levels. The dosimetry results must include the minimum absorbed dose, maximum absorbed dose, and the uniformity ratio. In addition, a description of the processing procedures should be provided (e.g., bulk density evaluations, number of boxes per transportation car, number of passes around the source, the positioning of the transporter cars within the chamber, and the total time product was exposed to the source). Calibration of dosimeters used shall be traceable to the National Bureau of Standards and shall be accurate to +/-5 percent. Weight of the product in a box should be uniform (e.g., 60 pounds). Temperature of the product at initiation of irradiation and after irradiation will be no more than 0-2°C and product will be held at this temperature until shipped to a laboratory for storage and analyses. Product in transit will be approximately 0-2°C.

Competent Laboratory

Storage Conditions for Each Variable:

° Irradiated and non-irradiated vacuum-packaged sliced loin samples will be stored at 5°C (refrigeration), 15°C (abuse), and 26°C (severe abuse). Product will be observed during storage for odors and appearance that are indicative of spoilage.

Frequency and Sampling of Each Variable During Storage (Maximum 35 days):

At 5°C storage - 5 samples of each variable will be pulled at 0, 12, 24, and 35 days for testing.

At 15°C storage - 5 samples of each variable will be pulled at 0 and every 48 hours up to 35 days, or until 5 of 5 units are clearly odor unacceptable.

At 26°C storage - 5 samples of each variable will be pulled at 0 and daily up to 35 days, or until 5 of 5 units are clearly odor unacceptable.

Analytical Procedures:

° MPN on each individual C. botulinum strain spore crop.

° MPN on the composite spore preparation.

° APC 35°, APC 20°, coliforms and E. coli, and pH on uninoculated stored samples. Pull intervals until 5/5 unacceptable is reached.

° Toxin testing of inoculated/vacuum-packaged product at each sampling time. Intraperitoneal injection of protected and unprotected mice (FDA-BAM).

Monitoring

To ensure that the research adheres to the critical variables outlined above, representatives from FSIS and FDA will review all study protocols and procedures (including lab QA) and will monitor all phases of the research. Agency representatives may visit the study facilities, if deemed necessary, according to the following:

° FSIS inspection - carcass breaking and slicing.

° FSIS/FDA - at botulinal inoculation.

° FSIS/FDA - at irradiation.

° FSIS/FDA - at toxin testing laboratory.

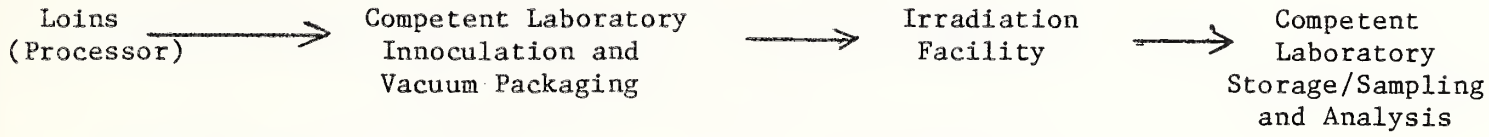
Other Research Areas of Interest

Simultaneous to this study, a processor may want to:

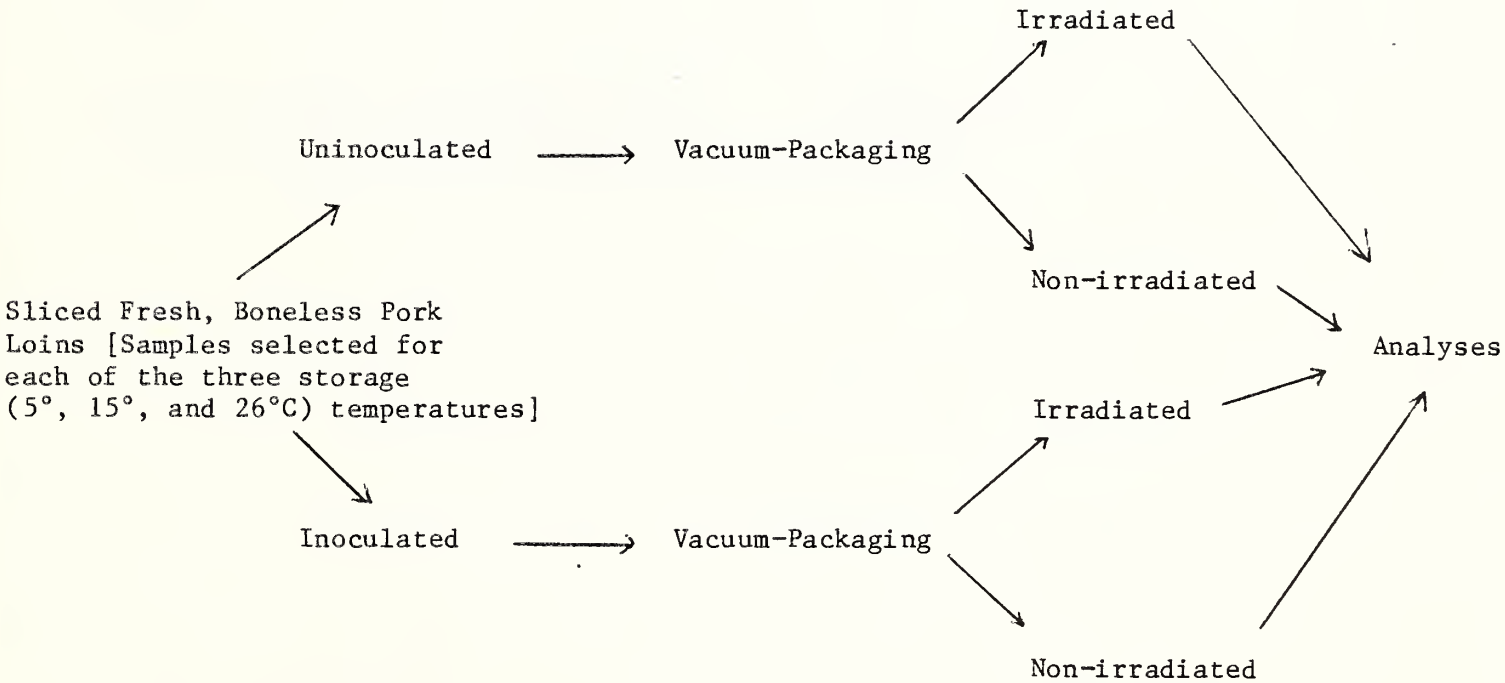
- Investigate the effects of irradiaton on pathogens such as Salmonella, Yersinia, Campylobacter, Listeria, Aeromonas and *E. Coli* 0157-H7.
- Investigate the use of temperature (abuse) indicators.
- Implement the FSIS partial Quality Control Program (PQC) guidelines for food irradiation facilities as a test-run.

Figure 1. STUDY SCHEME/SUGGESTED EXPERIMENTAL DESIGN

Sample Flow:



Sample Treatment:



Concurrence:

Robert C. Post, FIAD, Science — 6/12/86 Date

Ralph Johnston, Micro., Science — 6-19-86 Date

Dan Engeljohn, PPID, MPITS — 6/19/86 Date

Donald Kauter, Micro., FDA — 6/19/86 Date

Doug Archer, Micro., FDA — 6/19/86 Date

Approved:

Ronald E. Engel, Deputy Administrator for Science — 30 Jun 86 Date